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Short communication

Antimicrobial, anti-inflammatory and genotoxicity activity of *Alepidea amatymbica* and *Alepidea natalensis* (Apiaceae)

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Abstract

Antibacterial, antifungal, anti-inflammatory and genotoxicity tests were carried out on petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water extracts of two *Alepidea* species, *A. natalensis* Wood & Evans and *A. amatymbica* Eckl. & Zeyh. Water extracts of *A. natalensis* rhizomes exhibited high activity against four bacterial strains; *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* with MIC values of 0.78 mg/ml. High activity was also observed in the PE and DCM leaf extract of the same plant against the Gram-positive bacteria. The PE and DCM extracts of *A. amatymbica* rhizomes exhibited the best activity (MIC values of 0.39 mg/ml) against *B. subtilis*. The rest of the extracts showed low activity (MIC values>1 mg/ml). All the extracts showed activity against *Candida albicans*, with *A. natalensis* leaf extracts exhibiting the highest antifungal activity with MIC values of 0.88, 0.2 and 0.78 mg/ml for PE, DCM and EtOH respectively. The PE and DCM extracts had high COX-1 activity with percentage inhibitions above 70%. Ethanol extracts had inhibition less than 40% for both *A. natalensis* and *A. amatymbica*. All the PE extracts showed higher COX-2 inhibitory activity than for COX-1. There were no significant differences in the activity exhibited by DCM extracts in COX-1 and COX-2. PE and DCM extracts both had percentage inhibitions above 70% for both COX-1 and COX-2 inhibition. The Ames test for genotoxicity revealed that none of the plant extracts significantly increased the number of His+ revertants with respect to the level observed in the negative control plates.

Keywords: *Alepidea* species; Anti-inflammatory; Antimicrobial; Cyclooxygenase enzymes; Genotoxicity

1. Introduction

Alepidea F. Delaroche is a genus consisting of about 28 species restricted to southern and eastern Africa, from South Africa northwards to Kenya and Ethiopia (Klopper et al., 2006; Van Wyk et al., 2008). The genus belongs to the family Apiaceae and is placed in the subfamily Saniculoideae. The plants are characterised by simple leaves with markedly toothed and setacous or bristly margins, attractive pseudanthia with prominent involucral bracts, and sessile flowers. The shape, size and surface sculpturing of the fruits are used for taxonomic purposes (De Castro and Van Wyk, 1994). Seven of the 28 species (*A. amatymbica* Eckl. & Zeyh., *A. natalensis* Wood & Evans, *A. pilifera* Weimack, *A. longifolia* [E. Mey. Ex] Dümmer, *A. setifera* N.E.Br., *A. comosa* Dümmer, *A. macowanii* Dümmer) are known to be used for medicinal purposes (Hutchings, 1989; Van Wyk and Gericke, 2000; Maksimovic et al., 2004).

A. amatymbica is the most important medicinal plant in the genus and is the only one of all the *Alepidea* species that is widely sold in traditional markets as *ikhathazo* in KwaZulu-Natal and *lesoko* in Lesotho (Van Wyk et al., 2008). The rhizomes are used in traditional medicine for treatment of abdominal disorders, respiratory tract infections, colds and as a purgative (Hutchings, 1989; De Castro and Van Wyk, 1994).

The taxonomy of *Alepidea* is often confusing and is in an unsatisfactory state (De Castro and Van Wyk, 1994). The species look so similar that they are often wrongly identified, especially in traditional medicine. As *A. amatymbica* is scarce, it is easier for traditional herbalists to use other species of *Alepidea* and thus *A. natalensis* is often substituted. The purposes for which *A. natalensis* is largely harvested is still not clear (De Castro and Van Wyk, 1994). However, the leaf of the

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plant is used as a vegetable relish known as *ikhokwana* by the Zulu people. This study was undertaken to investigate the antibacterial, antifungal, anti-inflammatory and genotoxic effects of extracts of two *Alepidea* species, *A. natalensis* and *A. amatymbica*.

2. Materials and methods

2.1. Plant material

Plant materials were collected in March, 2007 from Mount Gilboa, KwaZulu-Natal, South Africa and separated into leaves and rhizomes. Voucher specimens [*A. natalensis* Wood & Evans. Mulaudzi 01 (NU) and *A. amatymbica* Eckl. & Zeyh. Mulaudzi 12 (NU)] were deposited in the University of KwaZulu-Natal Herbarium, Pietermaritzburg. The plant material was oven dried at 50 °C for 3 days, ground and stored in airtight containers at 10 °C in the dark.

2.2. Preparation of plant extracts

Dried, ground plant parts were extracted sequentially with 20 ml/g of PE, DCM, EtOH and water with sonication for 1 h, the temperature being kept low by adding ice to the water bath. The extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum using a rotary evaporator. The concentrated extracts were then dried at room temperature using a stream of cold air.

2.3. Antibacterial activity

Minimum inhibitory concentration (MIC) of two *Alepidea* species for antibacterial activity was determined using the micro-dilution bioassay in 96-well micro-plates (Eloff, 1998). One hundred microlitres of each resuspended sample (50 mg/ml) in 80% ethanol were two-fold serially diluted with sterile distilled water, in duplicate, down a 96-well micro-plate for each of the four bacteria employed. A similar two-fold serial dilution of neomycin (Sigma) (0.1 mg/ml) was used as a positive control against each bacterium. Water and bacteria-free broth were included as negative controls. Overnight cultures (incubated at 37 °C in a water bath with shaking) of four bacterial strains: two Gram-positive (*Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 12600) and two Gram-negative (*Escherichia coli* ATCC 11775 and *Klebsiella pneumoniae* ATCC 13883) were diluted with sterile Mueller-Hinton (MH) broth (1 ml bacteria/50 ml MH resulting in a final inoculum of approximately 10^6 cfu/ml). One hundred microlitres of each bacterial culture were added to each well. The plates were covered with parafilm and incubated overnight at 37 °C. Bacterial growth was tested by adding 50 µl of 0.2 mg/ml *p*-iodonitrotetrazolium chloride (INT) to each well and the plates incubated at 37 °C for 1 h. Bacterial growth in the wells was indicated by a red–pink colour, whereas clear wells indicated inhibition of growth by the tested sample. MIC values were recorded as the lowest concentration of extract showing a clear well. Each assay was repeated twice with two replicates.

2.4. Antifungal activity

The antifungal activity of the extracts was evaluated against *Candida albicans* (ATCC 10231) using the micro-dilution assay (Eloff, 1998) as modified by (Masoko et al., 2007). An overnight fungal culture was prepared in Yeast Malt (YM) broth. Sterile saline was added to 400 µl of a 24-h-old *C. albicans* culture to give approximately 10^6 cfu/ml (absorbance was read at 530 nm and adjusted to match that of a 0.5 M McFarland standard solution). From this prepared stock, a 1:1000 dilution with sterile YM broth was prepared. The assay was repeated twice with three replicates each.

2.5. Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the enzyme-based cyclooxygenase assays COX-1 and COX-2 as described by Zschocke and Van Staden (2000). Results are the mean of two experiments (each experiment in duplicate).

2.6. Genotoxicity test

Mutagenicity was tested using the *Salmonella* microsome assay based on the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA98. The assay was performed according to Maron and Ames (1983). Stock (100 µl) bacteria in 20 ml Oxoid No. 2 nutrient broth were incubated for 16 h at 37 °C. The bacterial cultures (100 µl) were added to 100 µl of plant extract in 500 µl phosphate buffer and 2 ml of agar containing biotin–histidine (0.5 mM). The mixture was poured on a minimal agar plate and incubated at 37 °C for 48 h. Samples were tested in triplicate. Three dilutions (50, 500, 5000 µg/ml of the sample) were used per sample. 4-Nitroquinoline-N-oxide (4NQO) was used as a positive control. Bacterial colonies were counted after 48 h.

2.7. Statistical analysis

Genotoxicity results were expressed as mean ± S.E.M. of three independent experiments and $P < 0.05$ was considered significant. The software used was the Graph Pad Prism Version 4.00 statistical software program for Windows (GraphPad Software Inc.).

3. Results and discussion

The antibacterial MIC values of the two *Alepidea* species are presented in Table 1. An antibacterial MIC less than 1 mg/ml was considered to be acceptable antibacterial activity (Aligiannis et al., 2001). The results indicate that the water extracts of *A. natanlensis* rhizomes contains compounds with high activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* despite reports that water extracts often do not have activity (Luseba et al., 2007). However, this was not the case for the leaf extracts of *A. natalensis* which showed low activity against all the bacteria strains used. Traditionally, water and not organic solvents is used to make decoctions. The *A. amatymbica* rhizome

Table 1
Antibacterial minimal inhibitory concentration (MIC) of extracts from *Alepidea* species.

Extract	MIC (mg/ml)											
	A. natalensis leaf				*A. natalensis* rhizome				*A. amatymbica* rhizome			
	B.s.	*E.c.*	*K.p.*	*S.a.*	*B.s.*	*E.c.*	*K.p.*	*S.a.*	*B.s.*	*E.c.*	*K.p.*	*S.a.*
PE	**0.78***	1.56	1.56	3.13	3.13	3.13	3.13	3.13	**0.39**	3.13	3.13	3.13
DCM	**0.78**	3.13	1.56	**0.78**	1.56	3.13	1.56	3.13	**0.39**	3.13	3.13	1.56
EtOH	3.13	3.13	1.56	3.13	3.13	3.13	**0.78**	3.13	1.56	**0.78**	**0.78**	1.56
Water	12.5	12.5	12.5	12.5	**0.78**	**0.78**	**0.78**	**0.78**	1.56	1.56	1.56	1.56

B.s.= Bacillus subtilis; E.c.= Escherichia coli; K.p.= Klebsiella pneumoniae; S.a.= Staphylococcus aureus.
*Values boldly-written are considered very active (<1 mg/ml).
The MIC values (μg/ml) for Neomycin (positive control) were: *B. subtilis*=1.6×10^{-3}; *E. coli*=0.8×10^{-3}; *S. aureus*=0.8×10^{-3}; *K. pneumoniae*=1.6×10^{-3}.

decoction is a popular remedy for colds and chest complaints (De Castro and Van Wyk, 1994). The activity observed in the water extracts of *A. natalensis* in our bioassays suggests that it is a good species to use in traditional medicine as a substitute for *A. amatymbica*.

The antifungal assay results as MIC and minimum fungicidal concentrations (MFC) are presented in Table 2. *A. natalensis* leaf extracts exhibited good antifungal activity with low MIC values for all extracts. It is important to check whether the active extracts were fungistatic or fungicidal. This was done by adding broth to all clear wells on the microtitre plate and incubating the plate for a further 24 h. The last clear well was then recorded as the MFC. It was noted that the DCM extract of *A. natalensis* leaf, PE and EtOH of *A. natalensis* and *A. amatymbica* rhizomes were fungicidal, as there was no change in the values of the last clear well after further addition of broth and 24 h incubation.

The ability of the extracts to inhibit prostaglandin synthesis by the extracts in COX-1 and COX-2 bioassays as percentage inhibition is shown in Fig. 1. Activity above 70% is considered as highly active (Taylor and Van Staden, 2001). All the PE and DCM had high COX-1 activity while the EtOH extracts for both *A. natalensis* and *A. amatymbica* had inhibition less than 40%. There were no significant differences in the percentage inhibition of COX-1 exhibited by the PE and DCM extracts of both *A. natalensis* and *A. amatymbica*. All the PE extracts showed higher COX-2 activity than COX-1. There were no significant differences on the activity exhibited by DCM extracts in COX-1 and COX-2. PE and DCM extracts both had percentage inhibitions above 70% for both COX-1 and COX-2 inhibition. High COX-1 activity is undesirable as it has been reported to cause damage to the gastrointestinal tract (Luseba et al., 2007). EtOH extracts showed higher COX-2 activity than COX-1 though the activity was moderate (<60%). Water extracts exhibited moderate activity (between 40% and 60%) for *A. natalensis* leaf and *A. amatymbica* rhizome for COX-1 inhibition, while the *A. natalensis* rhizome extract showed very low activity (<10%). All the water extracts showed very low activity (<20%) for COX-2 inhibition. It has often been reported that activity in water extracts is not detected or yields false positives (Luseba et al., 2007) possibly due to protein binding capability of phenolics. However, some phenolic

Table 2
Antifungal activity (MIC and MFC) of different extracts of two *Alepidea* species tested against *Candida albicans*.

Plant part	Extract	MIC (mg/ml)	MFC (mg/ml)
A. natalensis leaf	PE	**0.78***	1.56
	DCM	**0.20**	**0.20**
	EtOH	**0.78**	3.13
	Water	**0.78**	6.25
A. natalensis rhizome	PE	6.25	6.25
	DCM	3.13	6.25
	EtOH	6.25	6.25
	Water	1.56	1.56
A. amatymbica rhizome	PE	6.25	6.25
	DCM	1.56	1.56
	EtOH	6.25	6.25
	Water	**0.20**	1.56
Amphotericin B (μg/ml)		9.77×10^{-3}	7.81×10^{-2}

*Values boldly-written are considered very active (<1 mg/ml).

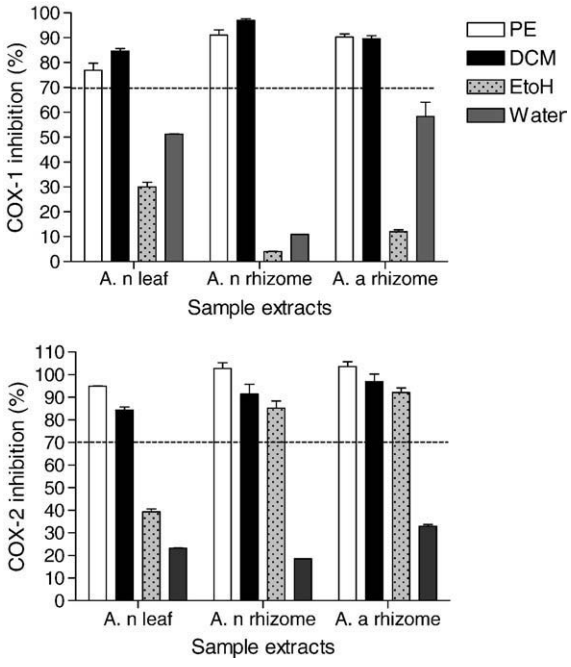

Fig. 1. Percentage inhibition of COX-1 and COX-2 enzymes by *A. natalensis* leaf (A. n leaf), *A. natalensis* rhizome (A. n rhizome) and *A. amatymbica* rhizome (A. a rhizome). Indomethacin® 5 μM for COX-1 and 200 μM for COX-2 positive control showed 60% and 64% inhibition respectively. Extracts with bar graphs above 70% were considered to be highly active. All the extracts were tested at 250 μg/ml.

Table 3
Number of His+ revertants in *Salmonella typhimurium* strain TA98 produced by *A. natalensis* and *A. amatymbica* leaf and root extracts.

Sample		Number of His+ revertants		
		5000 μg/ml	500 μg/ml	50 μg/ml
A. natalensis leaf	PE	23.7±0.3	19.7±1.8	21.3±0.7
	DCM	19.0±1.0	18.0±4.7	23.0±6.2
	EtOH	13.3±2.0	12.3±3.5	10.7± 0.9
A. natalensis rhizome	PE	22.0±3.5	19.0±2.0	21.0±0.6
	DCM	20.3±2.3	18.0±1.0	16.0±1.5
	EtOH	16.0±2.5	15.0±3.5	18.3±2.4
A. amatymbica rhizome	PE	20.3±2.8	23.0±1.5	22.3±3.2
	DCM	20.3±2.3	18.0±1.0	16.0±1.5
	EtOH	13.0±0.6	13.0±2.6	9.3±0.7
4NQO (+ve control, 2 μg/ml)			64.4±0.9	
Water (−ve control)			22.0±2.3	

Number of His+ revertants/plate: mean values of three triplicates, repeated three times.
+ve–positive.
−ve–negative.

compounds such as proanthocyanidins have been reported to have antioxidant effects and have been used to treat inflammatory diseases and wound healing (Ndhlala et al., 2008).

The rhizome of *A. amatymbica* contains high concentrations of diterpenoids of the kaurene type, the major compounds being dehydrokaurenoic acids and kaurenoic acids. The antimicrobial and anti-inflammatory activity of these two species could be attributed to the diterpenoids, although the compounds have not been tested individually (Hutchings, 1989; Van Wyk et al., 1997, 2008). The low activity values in some of the extracts tested in the present assays could be due to the impure form and/or low concentration of the active compound(s) in the crude extracts (Rabe and Van Staden, 1997).

The results of the Ames test which was used in this study to detect genetic damage, induced directly or indirectly, using *S. typhimurium* (TA98) are shown in Table 3. The results revealed that all the extracts were non-mutagenic towards *S. typhimurium* strain TA98. The average revertants observed ranged between 23.7 and 9.3 for all the extracts at all the concentrations whilst it was 22.0 for the negative control (water) and 64.4 for the positive control (4NQO). An extract was considered mutagenic if the number of revertants per plate was at least double that of the spontaneous revertants (negative control) (Bulmer et al., 2007).

4. Conclusions

A. natalensis which is not as popular as *A. amatymbica* in traditional medicine has both high antimicrobial and anti-inflammatory activity. The results revealed that *A. natalensis* leaves, which are normally used as a vegetable and not reported in traditional medicine, has potent antifungal and anti-inflammatory activity compared to the rhizome of the same plant and that of *A. amatymbica*. Both *A. natalensis* and *A. amatymbica* extracts did not yield genotoxic activity which suggests that these plants are probably safe for use in medicine, though other tests need to be conducted for further security. *A. natalensis* can be used as a conservation strategy to substitute for *A. amatymbica*.

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